FORM PTO-1390 (REV. 9-2001) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

| ATTORNEY 'S DOCKET NUMBER | |
|---|---|
| 31120-pa | |
| U.S. APPLICATION NO (If known, see 37 CFR 1 5 | |
| not yet assigned | 10/009417 |

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/US00/11865 | June 2, 2000 | June 4, 1999 |

TITLE OF INVENTION
Autologous Thrombin

APPLICANT(S) FOR DO/EO/US
Coelho, Philip H.; Kingsley, Phil; Brausch, Jim; Godsey, James H.; Rock, Gail; Madsen, Trista K.; Frausto, Sona B.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☑ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☑ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☑ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☑ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☑ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☑ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☑ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☑ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11 to 20 below concern document(s) or information included:**

11. ☑ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☑ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☑ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information:

| U.S. APPLICATION NO (if known, see 37 CFR 1.5) | INTERNATIONAL APPLICATION NO | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| not yet assigned 10/009417 | PCT/US00/11865 | 31120-pa |

| 21. ☑ The following fees are submitted: | CALCULATIONS PTO USE ONLY | |
|---|---|---|
| **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** | | |
| Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... **$1040.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... **$890.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... **$740.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **$710.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... **$100.00** | | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | $ 710.00 | |
| Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | $ | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
|---|---|---|---|---|---|
| Total claims | 17 - 20 = | 0 | x **$18.00** | $ | |
| Independent claims | 7 - 3 = | 4 | x **$84.00** | $ 336.00 | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | + **$280.00** | $ | |
| **TOTAL OF ABOVE CALCULATIONS =** | | | | $ 1,046.00 | |
| ☑ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. + | | | | $ (355.00) | |
| **SUBTOTAL =** | | | | $ 691.00 | |
| Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | $ | |
| **TOTAL NATIONAL FEE =** | | | | $ 691.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + | | | | $ 40.00 | |
| **TOTAL FEES ENCLOSED =** | | | | $ 731.00 | |
| | | | | **Amount to be refunded:** | $ |
| | | | | **charged:** | $ |

a. ☑ A check in the amount of $ 731.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-1734 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

Bernhard Kreten
Bernhard Kreten, Esq. & Associates
77 Cadilllac Drive Suite 245
Sacramento CA 95825

SIGNATURE

Bernhard Kreten
NAME

27,037
REGISTRATION NUMBER

PTO/SB/96 (08-00)
Approved for use through 10/31/2002. OMB 0651-0031
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## STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Coelho, et al.

Application No./Patent No.: 09/328,350 Filed/Issue Date: June 2, 2000

Entitled: Autologous Thrombin Biological Glue Processing Apparatus, Particularly for Thrombin and Method Therefore

ThermoGenesis Corp., a Delaware Corporation,
(Name of Assignee) (Type of Assignee, e g , corporation, partnership, university, government agency, etc )

states that it is:

1. [XX] the assignee of the entire right, title, and interest; or

2. [ ] an assignee of less than the entire right, title and interest.
The extent (by, percentage) of its ownership interest is _____%

in the patent application/patent identified above by virtue of either:

A. [x] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 010374, Frame 0488, or for which a copy thereof is attached.

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From:_____To:_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame_____, or for which a copy thereof is attached.

2. From:_____To:_____
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The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame_____, or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[ ] Copies of assignments or other documents in the chain of title are attached.
[NOTE: A separate copy *(i.e.,* the original assignment document or a true copy of the original document) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Nov 13, 2001
Date

Philip H. Coelho
Typed or printed name

Philip H. Coelho
Signature

Chief Executive Officer
Title

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

UNITED STATES PATENT AND TRADEMARK OFFICE

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|---|---|
| **APPLICANT:** | Coelho, P. et al. |
| **PRIORITY DATE:** | June 4, 1999 |
| **FOR:** | Autologous Thrombin |

To: Commissioner of Patents and Trademarks
Washington, DC 20231

## PRELIMINARY AMENDMENT

Sir:

Before a First Office Action on the merits, kindly enter the following amendments:

## IN THE CLAIMS

Kindly cancel claims 1 through 8, 14 through 16, 21, and 28 through 52 without prejudice or disclaimer as to their content.

**Kindly A mend the Claims as Follows:**

Claim 9 (amended) - Autologous thrombin, prepared using ethanol, which provides fast clotting in less than five seconds and is stable for more than fifteen minutes.

Claim 10 (amended) - A composition for extracting thrombin from plasma consisting essentially of:

unadulterated Plasma;

Ethanol (ETOH), present at a concentration between about 8% and about 20% volume per unit volume; and

$CaCl_2$.

Claim 13 (amended) - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 22 (amended) - A composition for extracting thrombin from plasma consisting essentially of:

plasma;

ethanol (ETOH), present at a concentration between about 8% and about 20% volume per unit volume;

$CaCl_2$; and

glass beads.

Claim 25 (amended) - The composition of claim 22 wherein $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM by volume in final concentration.

**Kindly add the new claim as follows:**

Claim 54 (new) - Thrombin prepared by a process consisting of the steps of:

using ethanol, at a concentration of about 8% to about 20% volume per unit volume, to sequester prothrombin from plasma taken from one person,

converting the prothrombin to thrombin, and

removing particulate material from the thrombin.

## REMARKS

This Preliminary Amendment is provided before receipt of any substantive Office Action on the merits in this case and is provided to rectify various minor

typographical inexactitudes and to present amended and new claims for the Examiner's kind consideration. No new matter has been presented.

In view of the foregoing, it is respectfully requested that the Examiner enter these amendments to this case.

Dated: December 4, 2001

Respectfully Submitted:

BERNHARD KRETEN
Applicant's Attorney
Telephone (916) 921-6181
Registration No.: 27,037

# AUTOLOGOUS THROMBIN

## Technical Field

The following invention relates generally to the preparation of a high specific activity thrombin enzyme from a given unit of plasma, which is sufficiently stable that it provides rapid clotting of a fibrinogen-rich solution of clotting and adhesive proteins for more than six hours when held at room temperature or lower.

## Background Art

Formulation of a fibrin sealant mimics the last step of the coagulation cascade wherein the enzyme thrombin cleaves fibrinogen which is then cross-linked into a semi-rigid or flexible fibrin clot. This fibrin clot adheres to wound sites, forming a barrier to fluid leaks and generates adhesion between tissues, while providing hemostatic and healing properties to the treated site.

Presently marketed, applicant's CryoSeal™ system is a device which harvests cryoprecipitated, concentrated clotting and adhesive proteins, including fibrinogen and Factor XIII from a donor's plasma in approximately one hour. The one hour cryoprecipitation harvesting, provided by the CryoSeal™ system, compared to the 1 to 2 day cryoprecipitation process routinely practiced in Blood Banks, means that CryoSeal™ harvesting of clotting and adhesive proteins can occur right in the perioperative theater with the patient close by, thereby avoiding the need to initiate the process days in advance.

These CryoSeal™ harvested clotting and adhesive proteins, when combined with bovine or human thrombin, forms a biological glue useful for surgical hemostasis and tissue adhesion. Commercially available thrombin, however, is generally sourced from bovine or human plasma pools, so the patient would still be at risk of negative immune reactions or contamination by infectious blood born viruses and, possibly Crutzfeld-Jacobs Disease (CJD) or new variants of CJD (NVCJD). An advantage of the CryoSeal™ cryoprecipitation invention is that the harvested clotting and adhesive proteins sourced from the patient's own blood eliminates the risk of contamination by infectious blood-borne disease when these

clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

The concept of utilizing thrombin and/or fibrinogen sourced from the patient in a medical procedure performed on that patient is not novel and was first described by Andrianova in 1974. Some twenty years later, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994 and its related U.S. Patent No. 5,795,780) describes an improved fibrin glue in which the thrombin component, which required thirteen steps, including centrifugation, and separation of intermediate precipitates and adjusting the ionic strength of the blood and pH of the plasma to prepare, would be combined with a fibrinogen component also sourced from the plasma of the same donor. However, these many preparation steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour. The present invention, *inter alia*, is distinguished in that it is undiluted by pH adjustment.

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192 and its related WO96/31245) follows Cederholm-Williams by teaching another method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which most of the fibrinogen in the plasma is first precipitated and removed to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different and simpler than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes. The present invention, *inter alia*, is distinguished in that the plasma is unprocessed as for example by not precipitating out fibrinogen.

These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Surgeons predominately require a fast acting clotting time (< 5 seconds) for hemostasis and tissue sealing or adhesion. Slow clotting biological glues (> 5 seconds) will often be transported away from the wound site by oozing and bleeding before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

The following prior art reflects the state of the art of which applicant is aware and is included herewith to discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

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| INVENTOR | PATENT NO. | ISSUE DATE |
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**OTHER PRIOR ART (Including Author, Title, Pertinent Pages, Date, Etc.)**

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The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

## Disclosure of Invention

The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (*e.g.* six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (*i.e.*, less than 5 seconds) during only a very narrow four to five minute time period, or required so many steps and elapsed time it would not be suitable for perioperative preparation, both totally impractical for the broad range of surgeries.

The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the use of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the use of commercial fibrin glues where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and

it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline, water and a dilute $CaCl_2$ solution (*e.g.* 125 mM $CaCl_2$) to provide precise, slower clotting times thereby allowing any preferred time from less than five seconds to longer than 2 minutes.

The procedure of the invention is preferably comprised of three steps, the first two of which should preferably occur at the same time:

1. Preparing a fraction enriched in prothrombin by use of an alcohol, preferably Ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Fibrinogen and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation to long-term functional thrombin.

2. Adding calcium ions to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.

3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

## Industrial Applicability

The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time in as little as thirty minutes, up to seventy-five minutes.

It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride ($CaCl_2$) to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

Viewed from a second vantage point, it is an object of the present invention to provide a method for generating autologous thrombin from a patient, the steps including: obtaining a blood product from the patient; sequestering plasma from the product; enriching the prothrombin in a plasma fraction; converting the prothrombin to thrombin, and filtering particulate from the thrombin.

Viewed from a third vantage point, it is an object of the present invention to provide a method for producing autologous thrombin which is stable for more than fifteen minutes, the steps including: sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

Viewed from a fourth vantage point, it is an object of the present invention to provide an autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

Viewed from a fifth vantage point, it is an object of the present invention to provide a composition for extracting thrombin from plasma consisting essentially of: Plasma; Ethanol (ETOH); $CaCl_2$.

Viewed from a sixth vantage point, it is an object of the present invention to provide a method for preparing thrombin comprising: obtaining plasma; adding ETOH and $CaCl_2$ to the plasma, forming a composition: agitating the composition;

incubating the composition in a static or rocking mode; filtering the composition of particulate, thereby passing the thrombin through the filter.

Viewed from a seventh vantage point, it is an object of the present invention to provide a device for preparing thrombin from plasma, comprising: a reaction chamber having a solution of $CaCl_2$ and ETOH therein; means for admitting plasma into the reaction chamber; thrombin receiving syringe coupled to the reaction chamber to receive the thrombin; and a filter located between the reaction chamber and the thrombin receiving syringe.

Viewed from an eighth vantage point, it is an object of the present invention to provide an autologous biological glue processing device, comprising, in combination: a thrombin processing means, a clotting and adhesive proteins processing means operatively coupled to the thrombin processing means, means for receiving plasma via the operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive proteins.

The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

The present invention further provides an efficient method of preparation. Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological components of the biological glue are easily combined in a surgical setting, administered to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing described herein provides a final product that is essentially free of contamination by non autologous microbes.

These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

## Brief Description Of Drawings

Figures 1A and 1B are perspective views of apparatuses for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

Figures 2A and 2B are plan views of the thrombin processing sets removed from the processing sets that extracts clotting and adhesive proteins.

Figures 3A and 3B are perspective views of the interior of the thrombin processing cases with the thrombin syringe shown in figures 2A and 2B removed therefrom.

Figures 4A and 4B are perspective views of the thrombin cases upper halves.

Figures 5A and 5B are perspective views of the thrombin cases lower halves.

Figures 6A and 6B are exploded parts views of the reaction chamber 26 shown in figures 3A and 3B along with the valving structure at opposed ends thereof.

Figures 7A and 7B are sectional views of the reaction chambers and valving structures depicted in figures 6A and 6B.

Figures 8A and 8B are detail of construction of that which is shown in figures 7A and 7B.

Figures 9A and 9B are exploded parts view of filter alternatives used in figures 3A and 3B.

Figure 10 is a perspective view of that which is shown in figure 9.

Figure 11 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations.

Figure 12 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations at different $CaCl_2$ concentrations.

Figure 13 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored on ice.

Figure 14 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored at room temperature.

Figure 15 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored on ice.

Figure 16 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored at room temperature.

**Best Mode(s) for Carrying Out the Invention**

Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figures 1A and 1B.

In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

More particularly, viewing both figures 1A and 1B, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has plural positions for stop valves 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60. The first valve branch 12 also includes a stop valve 6.

Viewing figure 1B, prior to the introduction of plasma through the first branch 12 thrombin processing unit 40, reagent from preloaded syringe 95 is injected pushing plunger mechanism 94 in the direction of A', into receiving system 20 through sterile barrier filter 92. The reagent passes through one way valve 91; Y connector 90, that merge coupling 18 and valve 91, through branch tubing 93; and finally into the interior of casing 22. Referring to figure 3B and 7B, a valve 24 initially directs the reagent to a reaction chamber 26.

Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. Although this system

can be dimensioned for any size batch, the plasma required for the thrombin processing unit will typically be 9-10 ml so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60.

In the embodiments shown in figures 1A and 1B, sealed bags 16 and 78 overlie both the thrombin dispensing syringe 42 (and a lead in of conduit 64) and cryoprecipitate storage tube 76 to provide sterility until both storage containers are introduced into a sterile surgical field (e.g., operatory). Prior to that, the thrombin processing unit 40 operates as shown and described with reference to figures 2A through 10. Viewing figure 1B, after reagent is added, plasma enters the first branch 12 and passes beyond a coupling 18, through tubing branch 93, and into an interior of the casing 22.

Referring back to figure 1A, the thrombin processing unit 40 operates as shown and described with reference to figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10. As mentioned, fluid enters the first branch 12 and (figure 1A) passes beyond a coupling 18 and into an interior of a casing 22. Coupling 18 is preferably frictionally and/or adhesively attached to the first branch 12 yet the thrombin processing unit 40 can still be removed (e.g. figure 2A) from the processing set 10 (e.g., by merely detaching or severing branch 12 followed perhaps with heat sealing) after receiving the plasma as shown in figure 2. If adhesive is used, it is a sterile grade for use in an operatory.

Referring to figure 3A, a valve 24 initially directs the plasma to a reaction chamber 26 having an interior tube 28a (figure 6A) preferably formed from glass and capable of receiving a volume, for example 15 ml. Glass tube 28a is preferably shorter than and circumscribed by an overlying barrel 32 preferably formed from PVC. A window 31a in the PVC barrel 32 can be used to gauge and/or verify the contents within the glass tube 28a. Gauging may also include gradations 29, indicating a volume on the glass tube. The glass tube 28a of the reaction chamber 26 receives the plasma from the first branch 12 and into its interior for mixing with reagents preloaded in the glass tube 28a and described hereinafter. As shown in figure 7A, the interior of the glass tube is preferably prefilled only partially with beads 25 preferably formed from borosilicate, glass or ceramic to enhance the reaction and agitation.

Referring to figure 3B, a valve 24 initially directs the plasma to a reaction chamber 26 having tube 28b (figure 6B) preferably formed from clear polycarbonate and capable of receiving a volume, for example, 15 ml. Graduated lines 31b on the polycarbonate tube 28b can be used to gauge the contents within the tube 28b. The polycarbonate tube 28b of the reaction chamber 26 receives the plasma from the first branch 12 and into the interior for mixing with reagents previously added into the polycarbonate tube 28b and described hereinafter. As shown in figure 7B, the interior of the tube 28b is preferably prefilled only partially with beads 25 preferably formed from borosilicate or ceramic to enhance the reaction and agitation.

The reaction chamber 26 of the embodiment shown in figures 1A and 3A is formed with first and second end caps 34 detailed in figures 6A, 7A and 8A. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 is isolated from the beads 25 by a screen 23 nested within necked-down portion 48. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the glass tube 28a and through flow is controlled by the valves 24, 44. As shown in figure 8A, the cap 34 includes an annular necked-down portion 48a which frictionally and/or adhesively resides within an interior hollow of the PVC barrel 32. In this way, the necked-down portion 48 rests upon ends of the glass tube 28a in sealing engagement therewith, isolating the interior of the reaction chamber from the PVC barrel 32.

For the embodiment forming the reaction chamber 26 of the embodiment shown in figures 1B and 3B mainly out of polycarbonate tube 28 is detailed in figures 6B, 7B and 8B. This reaction chamber 26 is formed with first and second end caps 34 detailed in figure 8B. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 has interior obstructions preventing passage of beads 25 while allowing passage of fluid. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a

respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the polycarbonate tube 28b and through flow is controlled by the valves 24, 44. As shown in figure 8B, the cap 34 includes an annular interior recess portion 48b which adhesively resides on the interior surface of the polycarbonate tube 28b.

Preferably, ethanol and calcium chloride are the reagents which have been preloaded into the reaction chamber 26 or within reagent syringe 95. Initially, both valves 24 and 44 are oriented so that reagents will not pass therebeyond to seal the chamber for the embodiment of figure 1A. Viewing figure 1B, initially valve 24 is oriented so plasma will not enter reaction chamber 26, and valve 44 is oriented to allow passageway between the reaction chamber 26 and the draw plunger 56. Referring back to figure 1A, after the plasma has been pumped into processing unit 60, valve 44 is turned to allow access to the draw plunger 56 and valve 24 is oriented to allow access between the passageway 21 and the reaction chamber 26. Slide clip 6 is opened with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1A, syringe ~~56~~ plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. Referring back to figure 1B, the reagent syringe 95 is attached to open end of sterile barrier filter 92. Plunger 94 is depressed to transfer reagent syringe through sterile barrier filter and passageway 93 to reaction chamber 26. Likewise to the figure 1A embodiment, the figure 1B, with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1B, the syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. In both embodiments syringe 56 includes a filter 62 located in the flow path. More specifically, the path 43 between valve 44 and syringe 56 includes a filter 62 located in the flow path. The filter 62 provides an aesceptic microbial barrier so that, upon subsequent delivery of the thrombin to the dispensing syringe 42 (figure 1), there is no contamination from around the seal 57 of plunger 58 delivered to syringe 42. Plasma will subsequently enter chamber 26 from conduit 4 to replace air. Valve 24 is oriented to address filter 66. The reagents and plasma are briefly agitated assisted by beads 25 (and allowed to incubate for about 40 to 70 minutes). After incubation, thrombin processing unit 40 is agitated to loosen and break up gel formation. For the embodiment of figure 1B, the thrombin processing unit 40 is then returned to a motionless horizontal position for no less

than 10 minutes. Afterwards the thrombin processing unit 40 is again agitated to loosen and break up gel formation. For both embodiments, the plunger of syringe 56 is pushed in the direction opposite arrow A to move thrombin from chamber 26 through filter 66 into syringe 42. Delivery of thrombin to syringe 42 can be enhanced by retracting plunger 43 of syringe 42, defining a push pull system. Filter 66 removes particulate matter from the thrombin, including gel.

By allowing the thrombin contained in the reaction chamber 26 to reside therein after agitation for no less than 10 minutes enhances the effectiveness of the filter 66 in removing particulate matter for subsequent utilization. The time span for conversion and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer, or expression through a thin tube.

Figures 9A, 9B and 10 reveal alternative embodiments of filter 66 which includes an outer cylindrical wall 65 with end caps 34 each having a cylindrical spout 37 circumscribed by an annular recess 39. The alternative embodiment shown in figure 9A shows the centrally disposed cylindrical filter element 67a is preferably formed from polyurethane foam. While as shown in figure 9B the centrally disposed cylindrical filter element 67b is preferably formed from rolled polyester. Also shown in figure 9B, are circular filters 68 preferably formed from glass fiber or polyester. In each alternative embodiment, filter 67a or 67b filters by weight, size and protein binding.

Referring back to figures 1A and 1B, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 72 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 74 of the chamber 72 for subsequent extraction by means of a clotting and adhesive protein collection tube or dispensing syringe 76 contained in a sterile pouch 78. Chamber 72 is protected during this process by a filter vent 82 preventing contamination. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive collection tube or dispensing syringe 76, the two storage containers 42, 76 can be decoupled from the

processing set 10 (e.g. sterile disconnect device), and passed near the sterile, surgical arena. The overwrap bags are subsequently opened, and the storage containers 42, 76 are decoupled and transferred into the surgical area where the contents are dispensed into individual sterile 3cc plastic syringes which are subsequently loaded into the fibrin glue applicator for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

Both dispensing syringes 42 and 76 are stored at room temperature, or preferably stored at their optimal conditions: cryoprecipitate 76 being stored at room temperature and thrombin 42, stored in an ice bath at 1°C to 5°C. Please see figures 13 through 16.

Assume 9-10 ml of room temperature plasma is introduced into the reaction chamber 26. Other plasma volumes have utility. Please see figures 15 and 16. Add 1.0 ml of 75 mM calcium chloride ($CaCl_2$) and 2.0 ml of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and added to comprise 18.9% volume/unit volume or 15.02% ethanol weight/unit volume). Other ratios of reagent volume, comprising of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and a stock solution of 75 mM calcium chloride ($CaCl_2$)), to plasma volume have utility phase. Please see figures 13 and 14. The thrombin life span is shown to have been at least 300 minutes while its clotting time is at 2.98 seconds. An ethanol final concentration range between 8.0% and 20.0% (volume/unit volume), however, still has utility. Please see figure 11.

When the ethanol is at a final concentration of 18.9% volume/unit volume (as above) and the calcium chloride final concentration is 5.7 mM (1 ml taken from a 75 mM stock solution of calcium chloride), the thrombin lifespan also extends to at least 360 minutes while maintaining a clot time of 5.98 seconds when thrombin is stored at room temperature. Storing thrombin in optimal 1°C to 5°C ice bath typically maintains lot times of 2 to 3 seconds at 360 minutes. Calcium chloride stock solution concentrations ranging between 50 mM and 250 mM, however, have utility. Please see figure 12. The final concentrations range from 4.5mM to 23 mM.

Solutions such as saline, dilute $CaCl_2$ (e.g. 40mM to 125 mM $CaCl_2$) or even sterile water added to the thrombin can alter both the clotting time and life span of the thrombin. Assume an ethanol final concentration of 18.9% and a final calcium

chloride concentration of 5.7 mM was used in the reaction chamber 26. When the thrombin has been diluted 1 to 1.5 with water, the clot time has been extended to just less than 30 seconds, and has a life span of up to 150 minutes.

Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

## Claims

We Claim:

Claim 1 - A method for generating autologous thrombin from a patient, the steps consisting of:

obtaining a blood product from the patient;

sequestering unadulterated plasma from the blood product;

adding ethanol to the plasma to prepare a solution containing prothrombin;

converting the prothrombin in the solution to thrombin;

filtering the thrombin to remove particulate matter; and

applying the thrombin to the patient.

Claim 2 - The method of claim 1 further including the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 4 - The method of claim 2 wherein the converting step includes adding a source of calcium ions.

Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining unadulterated plasma.

Claim 6 - The method of claim 2 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes diluting the thrombin with any of the group consisting of saline, $CaCl_2$ solution and sterile water.

Claim 7 - The method of claim 6 including filtering the plasma by weight, size and protein binding.

Claim 8 - A method for producing fast clotting autologous thrombin which is stable for more than fifteen minutes, the steps consisting of:

using ethanol to sequester prothrombin from unadulterated plasma and converting the prothrombin to thrombin.

Claim 9 - Autologous thrombin, prepared using ethanol, which provides fast clotting in less than five seconds and is stable for more than fifteen minutes.

Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

unadulterated Plasma;

Ethanol (ETOH);

$CaCl_2$.

Claim 11 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 23.0 mM both by volume in final concentration.

Claim 12 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 5.7 mM both by volume in final concentration.

Claim 13 - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 14 - A method for preparing thrombin consisting essentially of:

obtaining unadulterated plasma;

adding ETOH and $CaCl_2$ to the unadulterated plasma, forming a composition:

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 15 - The method of claim 14 whereby subsequent to agitating the composition, incubating the composition for an amount of time greater than or equal to ten minutes.

Claim 16 - The method of claim 15 whereby prior to filtering the composition, re-agitating the composition.

Claim 17 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having a solution of $CaCl_2$ and ETOH therein;

means for admitting unadulterated plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 18 - A single donor biological glue processing device, comprising, in combination:

a thrombin processing means,

a clotting and adhesive proteins processing means operatively coupled to said thrombin processing means,

means for receiving plasma via said operative coupling for subsequent conversion of the plasma to, respectively thrombin in said thrombin processing means and clotting and adhesive proteins in said clotting and adhesive proteins processing means.

Claim 19 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having ceramic beads or borosilicate glass therein;

means for admitting a reagent into said reaction chamber;

means for admitting plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 20 - The device of claim 19 wherein the reagent includes $CaCl_2$ and ETOH solution.

Claim 21 - The method of claim 1 further including the step of contacting the plasma with glass beads.

Claim 22 - A composition for extracting thrombin from plasma consisting essentially of:

plasma;

ethanol (ETOH);

$CaCl_2$; and

glass beads.

Claim 23 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 23.0 mM both by volume in final concentration.

Claim 24 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 5.7 mM both by volume in final concentration.

Claim 25 - The composition of claim 22 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 26 - An apparatus to prepare thrombin from plasma consisting of:

a reacting chamber to accept $CaCl_2$ and ethanol, and means for delivery of plasma into said reacting chamber;

a syringe connected to said reacting chamber to receive thrombin from said reacting chamber;

and a filter between said reacting chamber and syringe which is to receive thrombin.

Claim 27 - The apparatus of claim 26 further including glass beads in said reacting chamber.

Claim 28 - A method for generating and then dispensing thrombin, the steps consisting of:

taking whole blood from a person,

sequestering prothrombin from the whole blood, using ethanol,

converting the prothrombin to thrombin,

loading the thrombin into a syringe, and

using the syringe to dispense the thrombin to stem blood flow.

Claim 29 - The method of claim 28 including loading clotting proteins into another syringe and dispensing the clotting proteins concurrently with the thrombin.

Claim 30 - A method for generating thrombin from one person, the steps consisting of:

using ethanol to sequester prothrombin from plasma taken from one person,

converting the prothrombin to thrombin, and

removing particulate material from the thrombin.

Claim 31 - The method of claim 30 further including diluting the thrombin to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 32 - The method of claim 31 including adding a source of calcium ions to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 33 - The method of claim 32 including adding $CaCl_2$ to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 34 - The method of claim 31 including adding saline to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 35 - The method of claim 31 including adding sterile water to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 36 - The method of claim 2 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding a source of calcium ions.

Claim 37 - The method of claim 2 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding $CaCl_2$.

Claim 38 - The method of claim 2 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding saline.

Claim 39 - The method of claim 2 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding sterile water.

Claim 40 - A method for generating thrombin from one person, the steps consisting of:

taking whole blood from one person,

obtaining plasma from the whole blood,

adding ethanol to the plasma to prepare a solution containing prothrombin,

converting the prothrombin to thrombin, and

sequestering the thrombin.

Claim 41 - The method of claim 40 further including the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot to a time of between about 2 seconds and about 5 seconds.

Claim 42 - The method of claim 41 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding a source of calcium ions.

Claim 43 - The method of claim 42 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding $CaCl_2$.

Claim 44 - The method of claim 41 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding saline.

Claim 45 - The method of claim 41 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding sterile water.

Claim 46 - The method of claim 40 including making the thrombin stable for a period of time between about fifteen minutes and three hundred and sixty minutes.

Claim 47 - The method of claim 46 including adding a source of calcium ions to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 48 - The method of claim 47 including adding $CaCl_2$ to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 49 - The method of claim 46 including adding saline to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 50 - The method of claim 46 including adding sterile water to alter the time required for the thrombin to convert fibrinogen to a fibrin clot.

Claim 53 - The device of claim 18 including a thrombin syringe coupled to said thrombin processing means to receive thrombin therefrom, said thrombin syringe initially ensconced in a bag, and

a clotting and adhesive protein syringe coupled to said clotting and adhesive protein processing means to receive clotting and adhesive proteins therefrom, said clotting and adhesive protein syringe initially ensconced in a bag.

Claim 54 - A method for generating autologous thrombin from a patient, the steps consisting essentially of:

obtaining a blood product from the patient;

sequestering plasma from the blood product;

adding ethanol to the plasma to prepare a solution containing prothrombin;

converting the prothrombin in the solution to thrombin;

filtering the thrombin to remove particulate matter; and

applying the thrombin to the patient.

Claim 55 - A method for generating and then dispensing thrombin, the steps consisting essentially of:

taking whole blood from a person,

sequestering prothrombin from the whole blood, using ethanol,

converting the prothrombin to thrombin,

loading the thrombin into a syringe, and

using the syringe to dispense the thrombin to stem blood flow.

Claim 56 - A method for generating thrombin from one person, the steps consisting essentially of:

using ethanol to sequester prothrombin from plasma taken from one person,

converting the prothrombin to thrombin, and

removing particulate material from the thrombin.

Claim 57 - A method for generating thrombin from one person, the steps consisting essentially of:

taking whole blood from one person,

obtaining plasma from the whole blood,

adding ethanol to the plasma to prepare a solution containing prothrombin,

converting the prothrombin to thrombin, and

sequestering the thrombin.

Claim 58 - The method of claim 57 wherein ethanol is present at a concentration between about 8% and about 20% per volume per unit volume.

Claim 59 - The method of claim 58 wherein ethanol is present at a concentration of about 18.9% volume per unit volume.

Claim 60 - The method of claim 57 wherein the time required to generate the thrombin is between about 30 minutes and about 75 minutes.

Claim 61 - The method of claim 57 wherein the time required to generate the thrombin is less than about one hour and greater than zero minutes.

Claim 62 - The method of claim 57 wherein the converting step includes adding $CaCl_2$.

Claim 63 - The method of claim 57 further including the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot to a time of between about two seconds and about five seconds.

Claim 64 - The method of claim 63 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding a source of calcium ions.

Claim 65 - The method of claim 64 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding $CaCl_2$.

Claim 66 - The method of claim 63 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding saline.

Claim 67 - The method of claim 63 wherein the step of altering the time required for the thrombin to convert fibrinogen to a fibrin clot includes adding sterile water.

Claim 68 - The method of claim 57 including making the thrombin stable for a period of time between about 15 minutes and about 360 minutes.

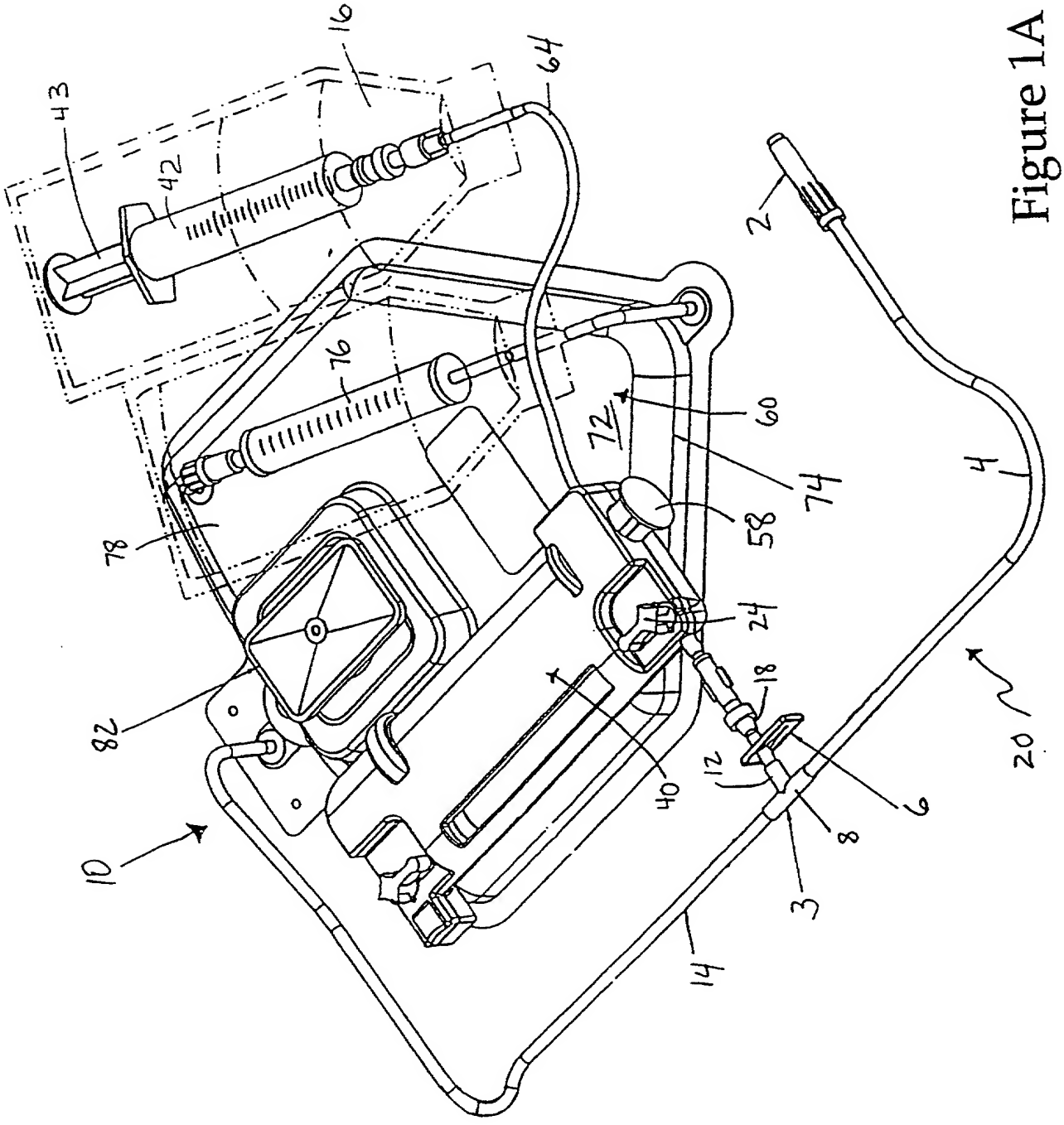
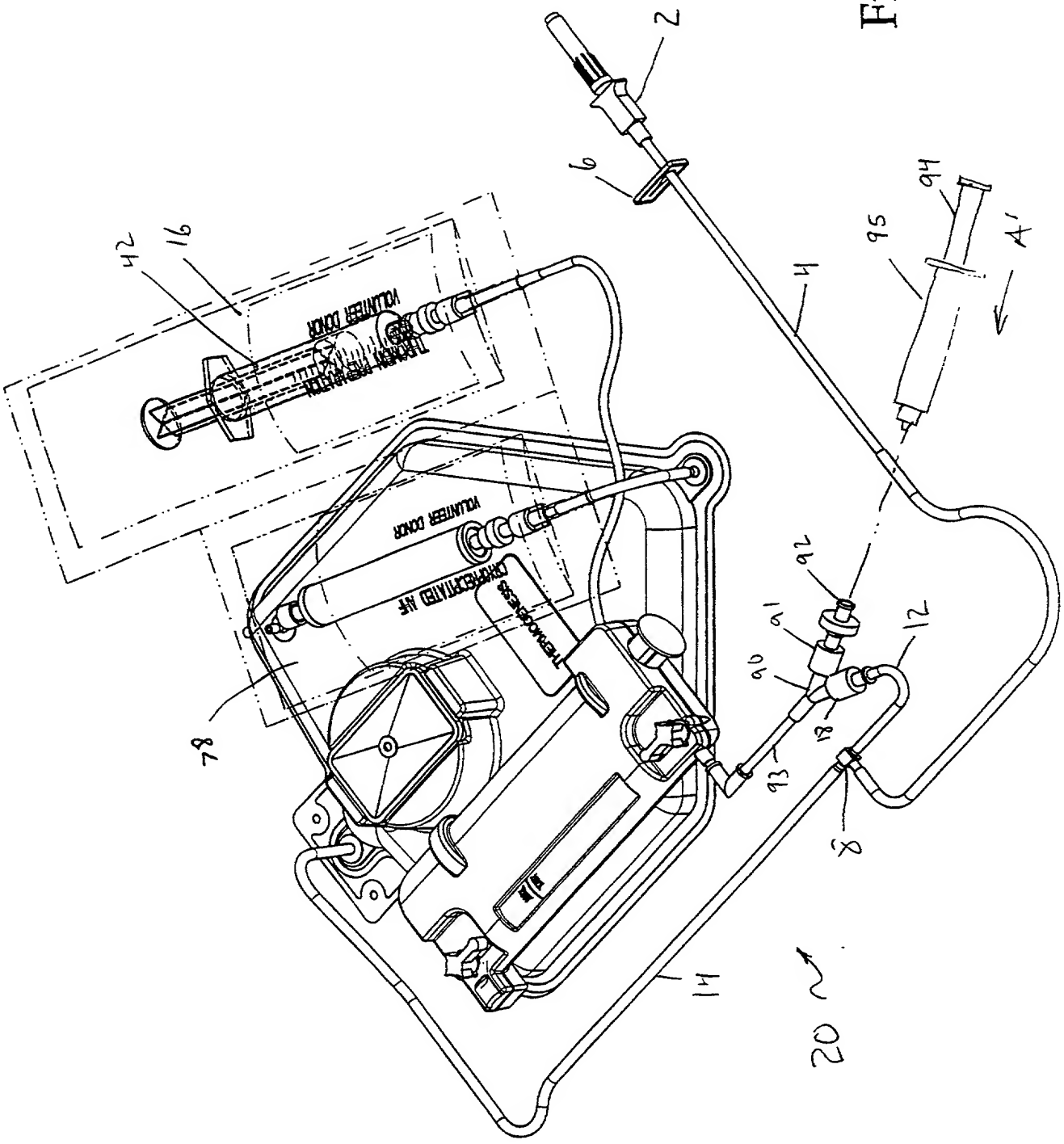



Figure 1A

Figure 1B



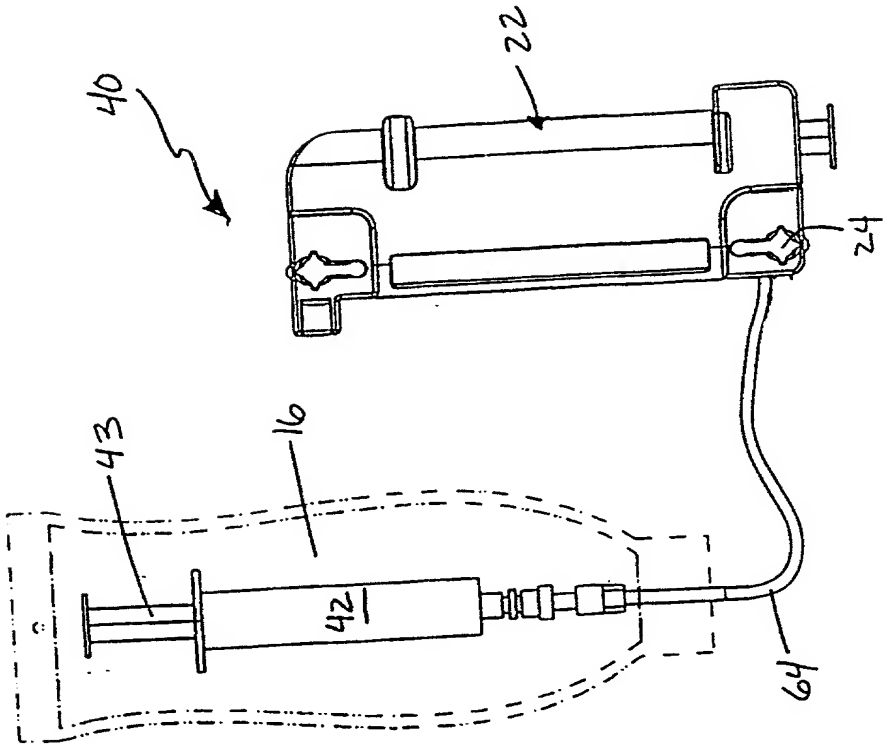



Figure 2B

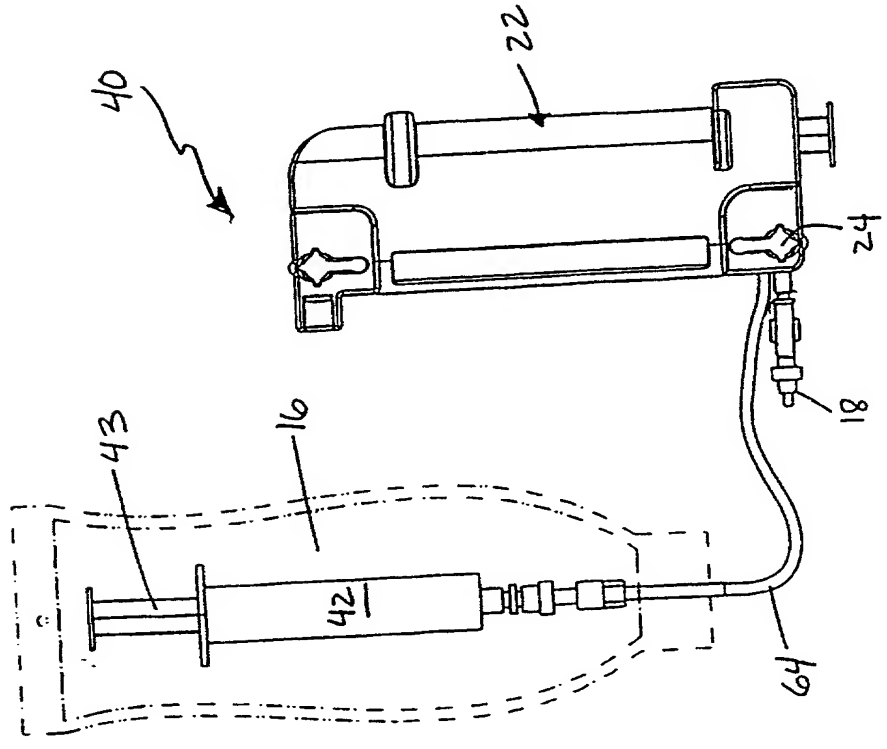



Figure 2A

10/009417
PCT/US00/11865



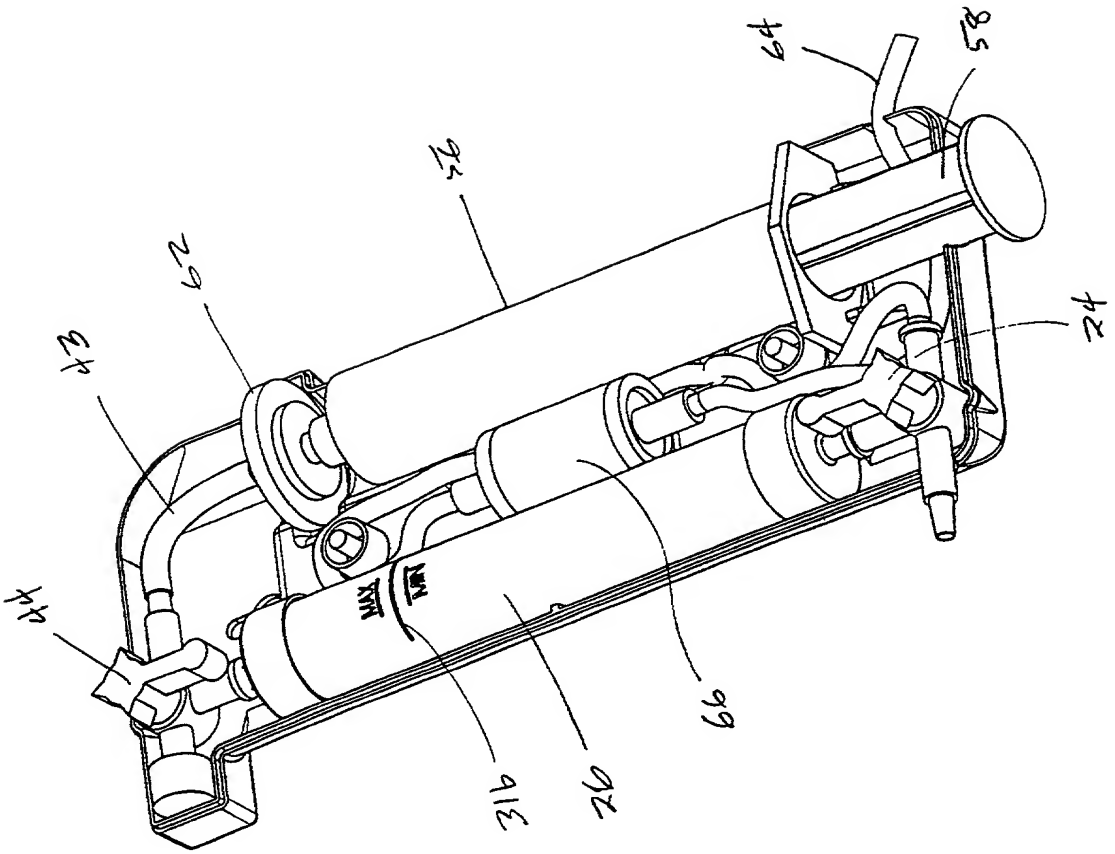



Figure 3B

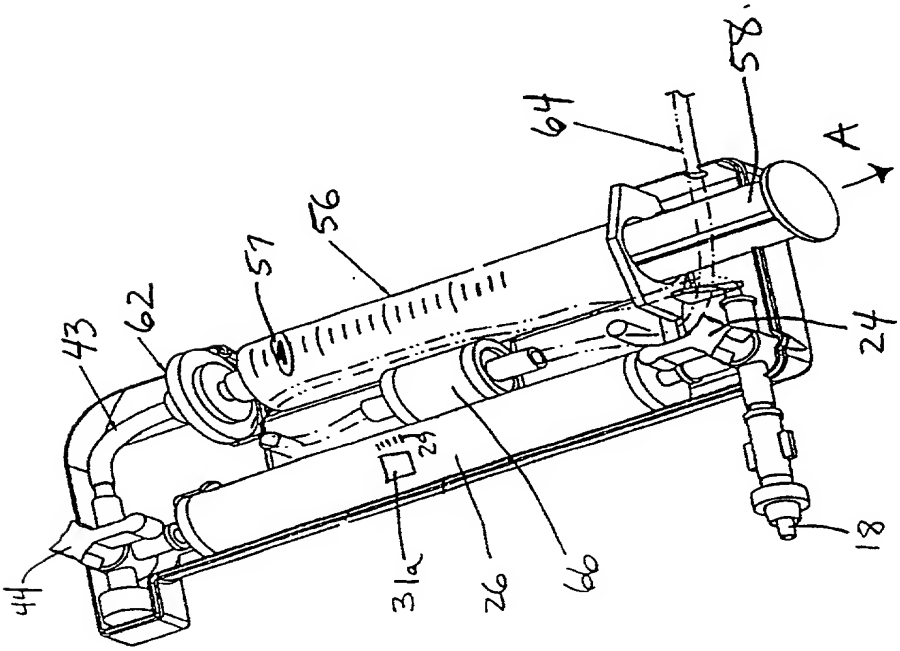



Figure 3A

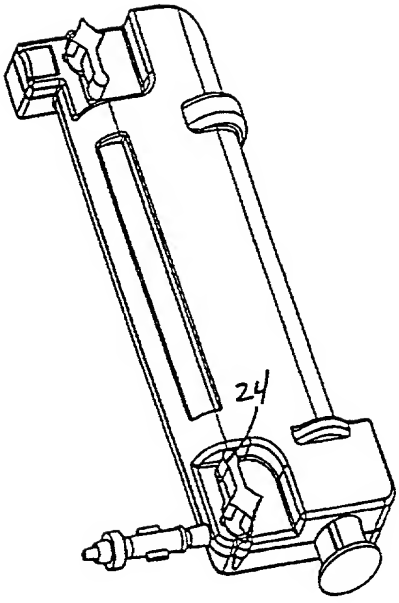



Figure 4A

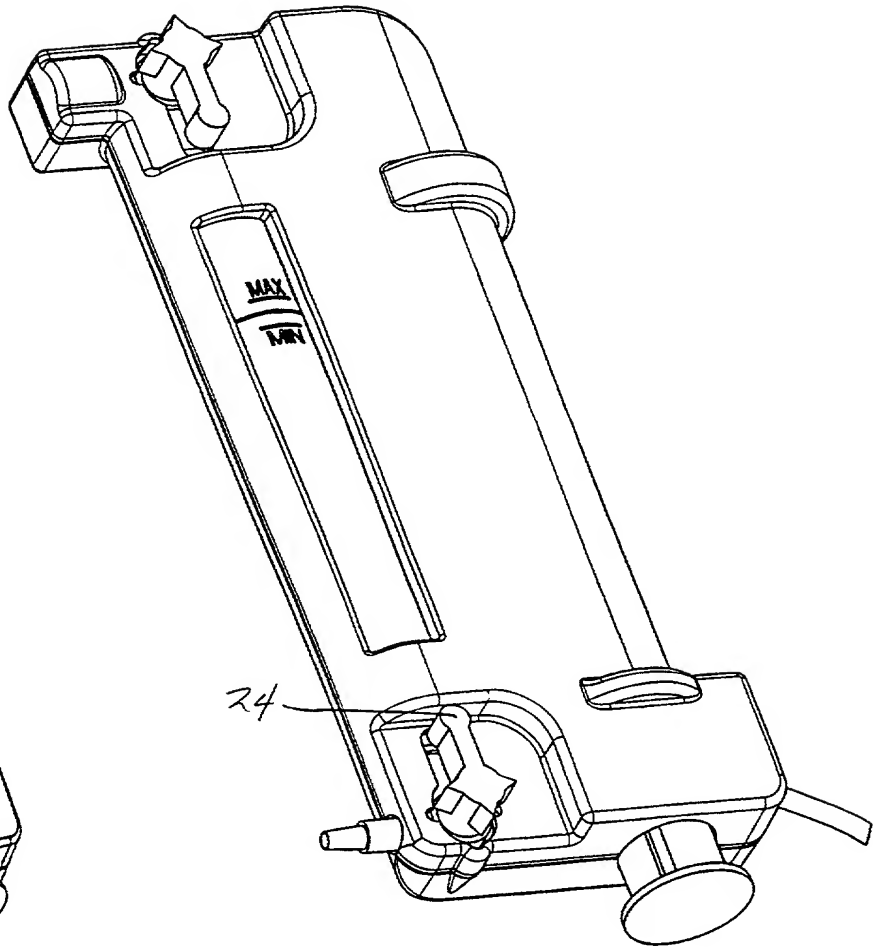



Figure 4B

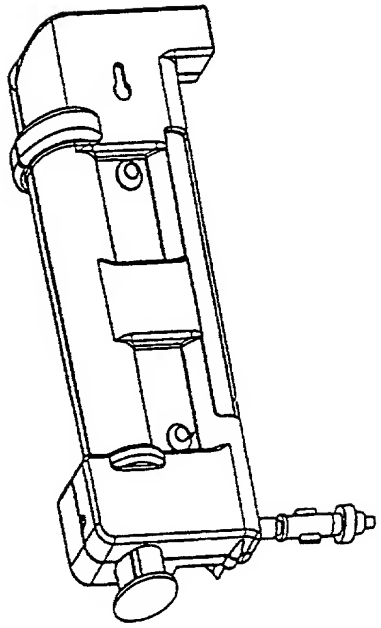


Figure 5A

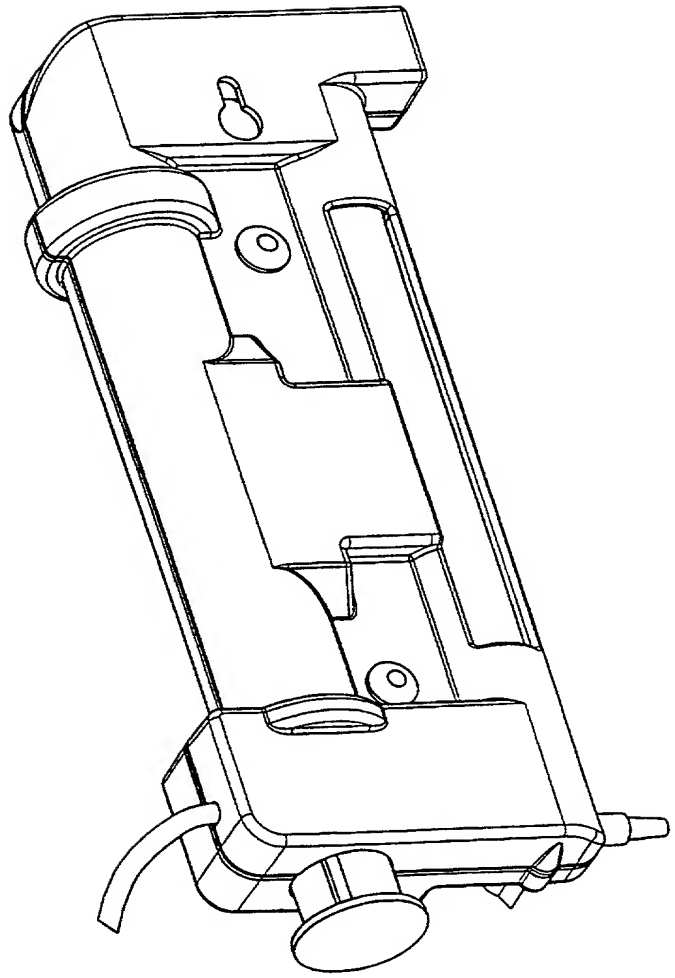


Figure 5B

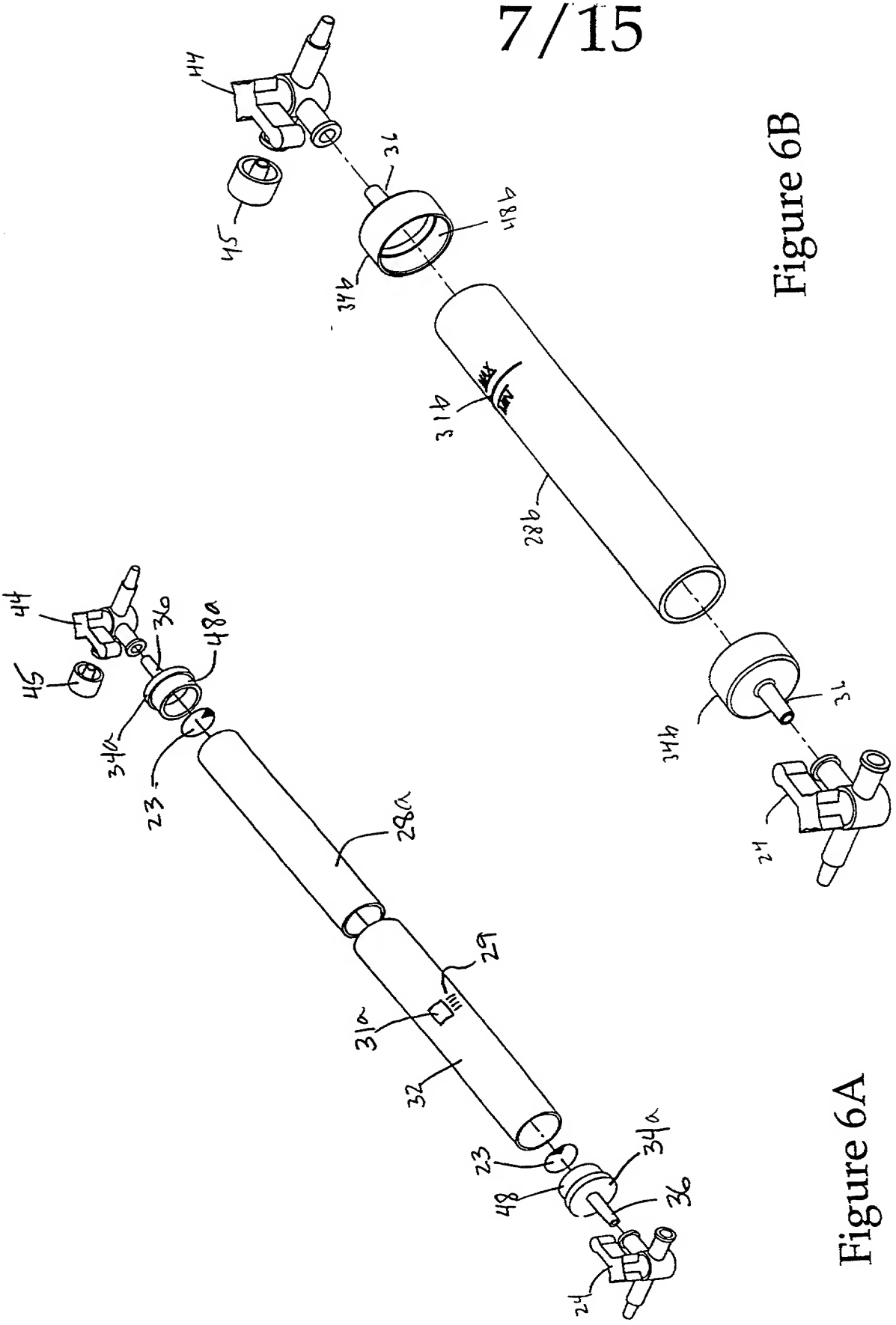



Figure 6B

Figure 6A

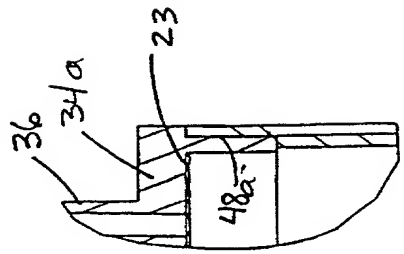



Figure 8A

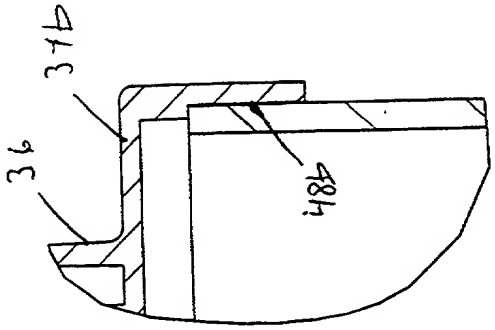



Figure 8B

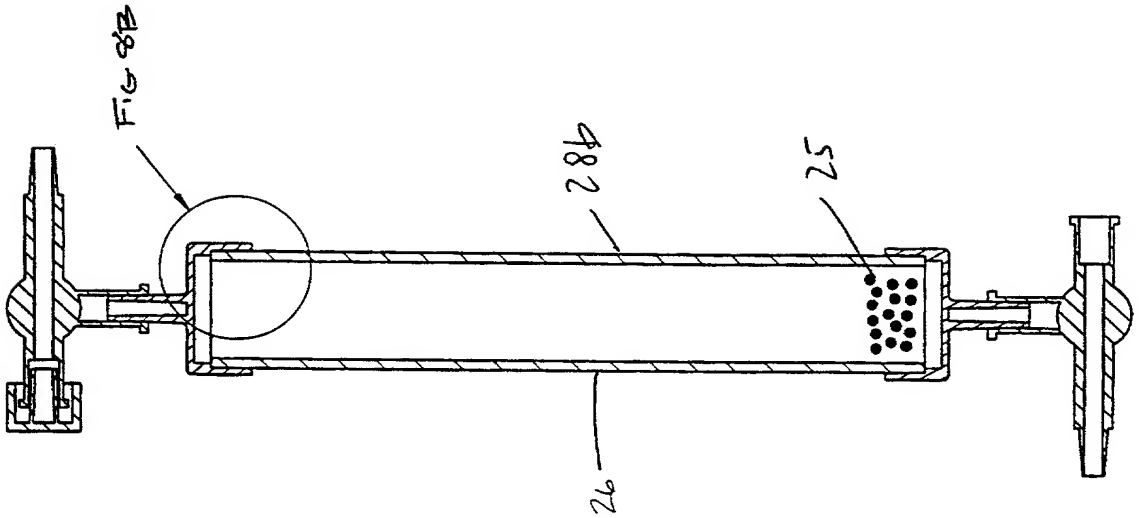



Figure 7B

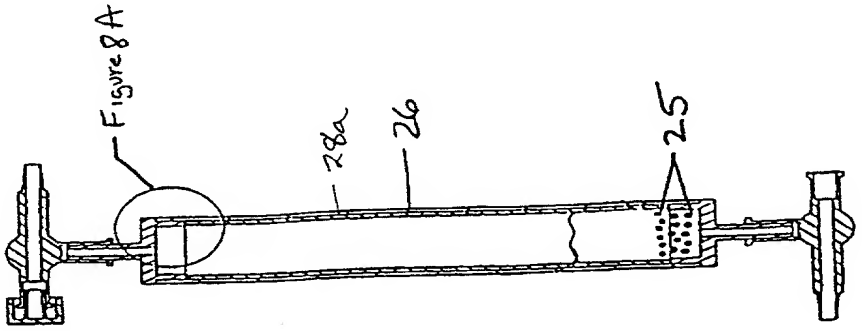



Figure 7A

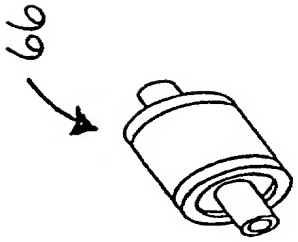



Figure 10

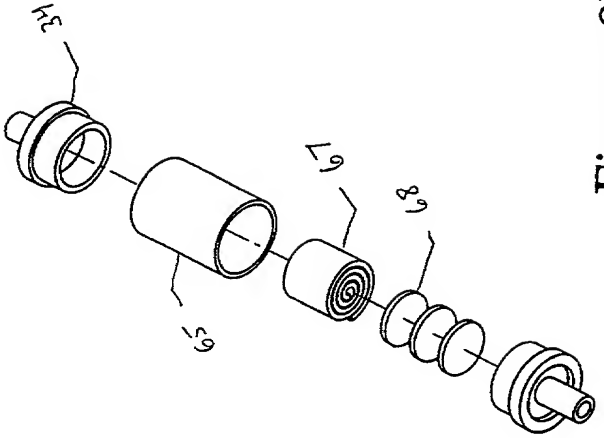



Figure 9B

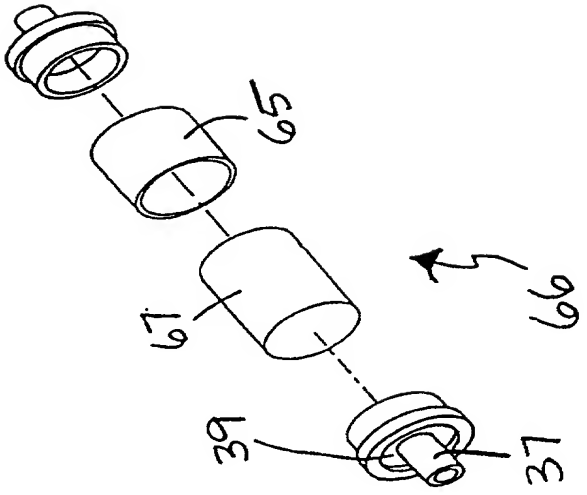



Figure 9A

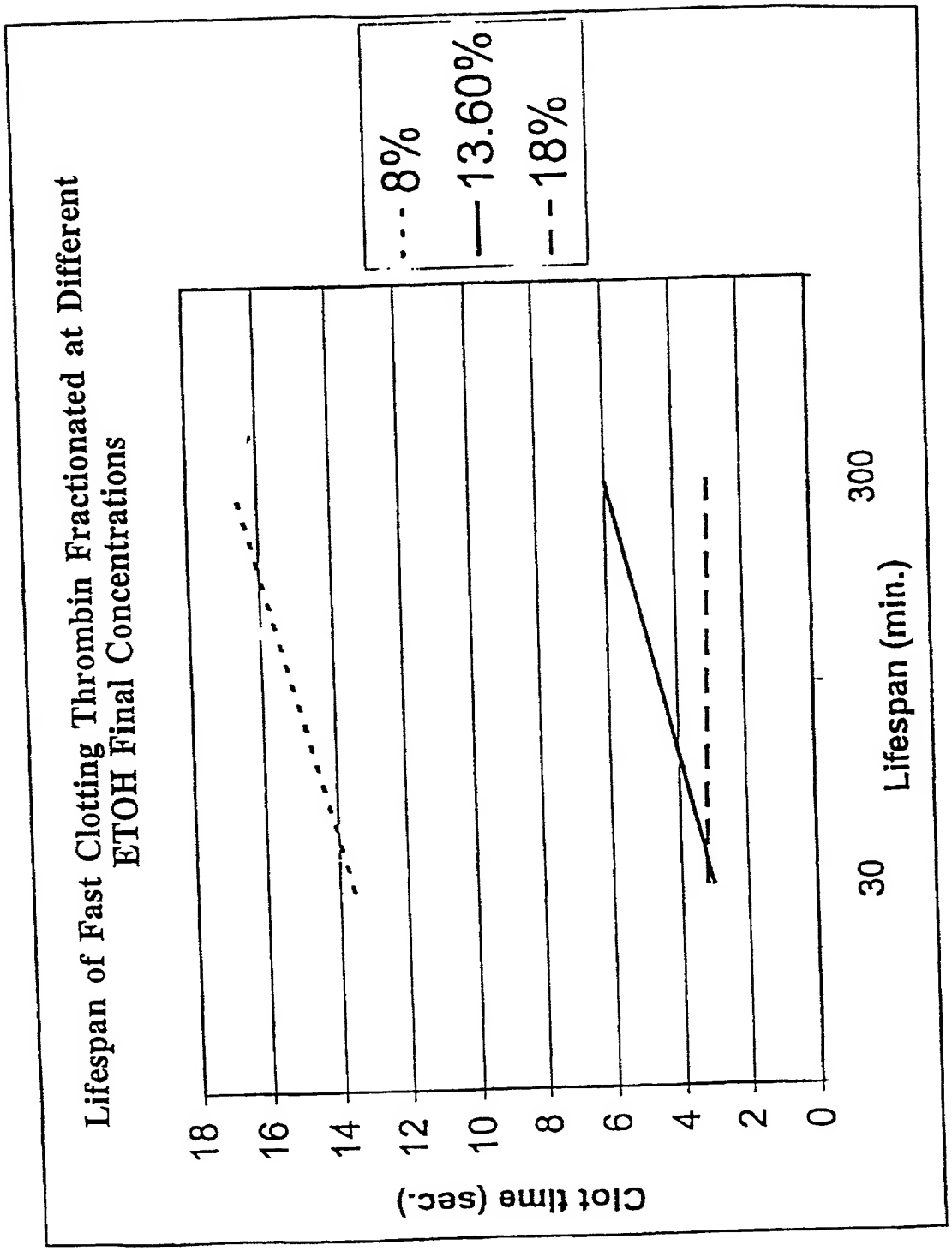

Lifespan of Fast Clotting Thrombin Fractionated at Different ETOH Final Concentrations
8%
13.60%
18%
Clot time (sec.)
0
2
4
6
8
10
12
14
16
18
30
300
Lifespan (min.)

Figure 11

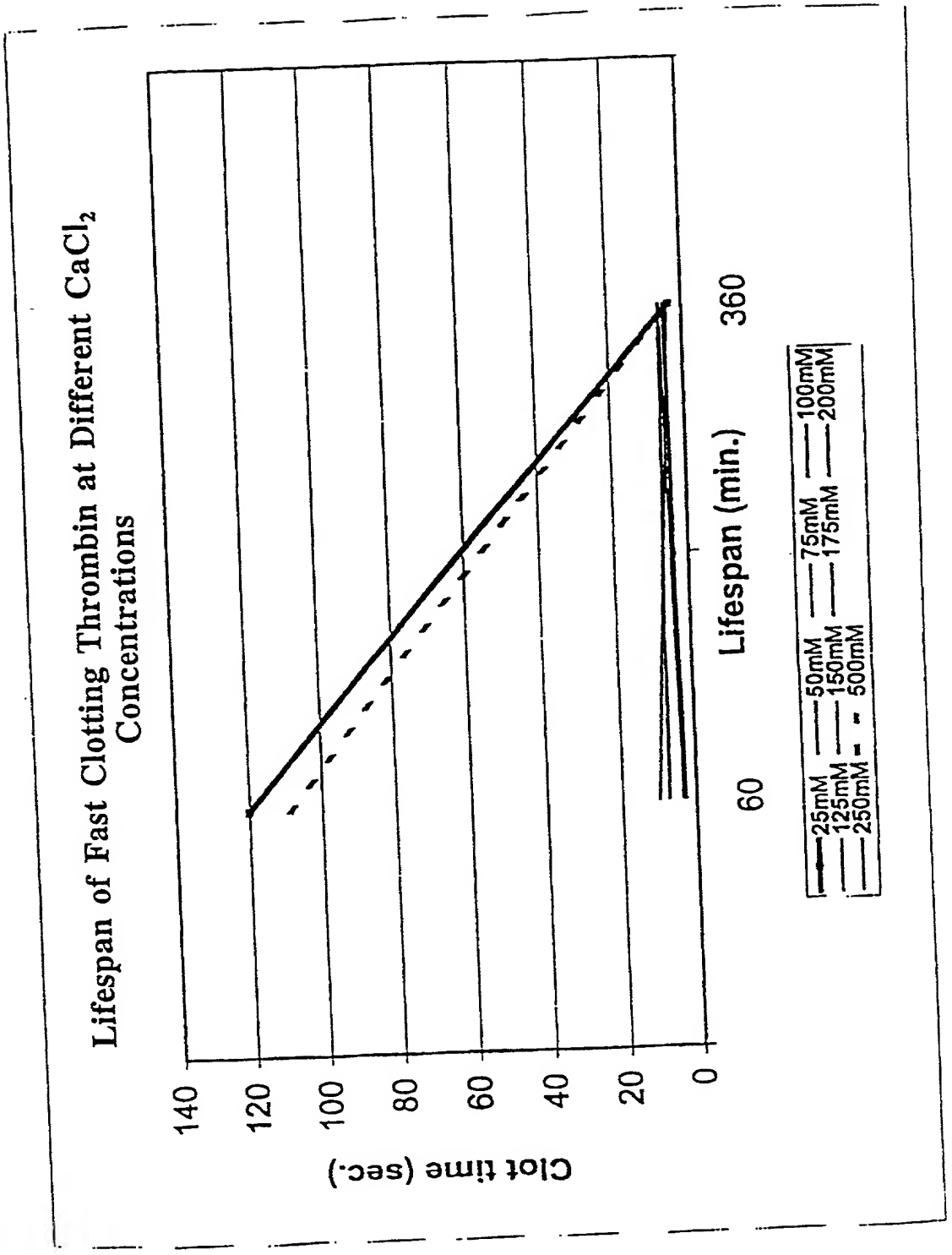

Lifespan of Fast Clotting Thrombin at Different $CaCl_2$ Concentrations
Clot time (sec.)
140
120
100
80
60
40
20
0
60
Lifespan (min.)
360
25mM
50mM
75mM
100mM
125mM
150mM
175mM
200mM
250mM
500mM

Figure 12

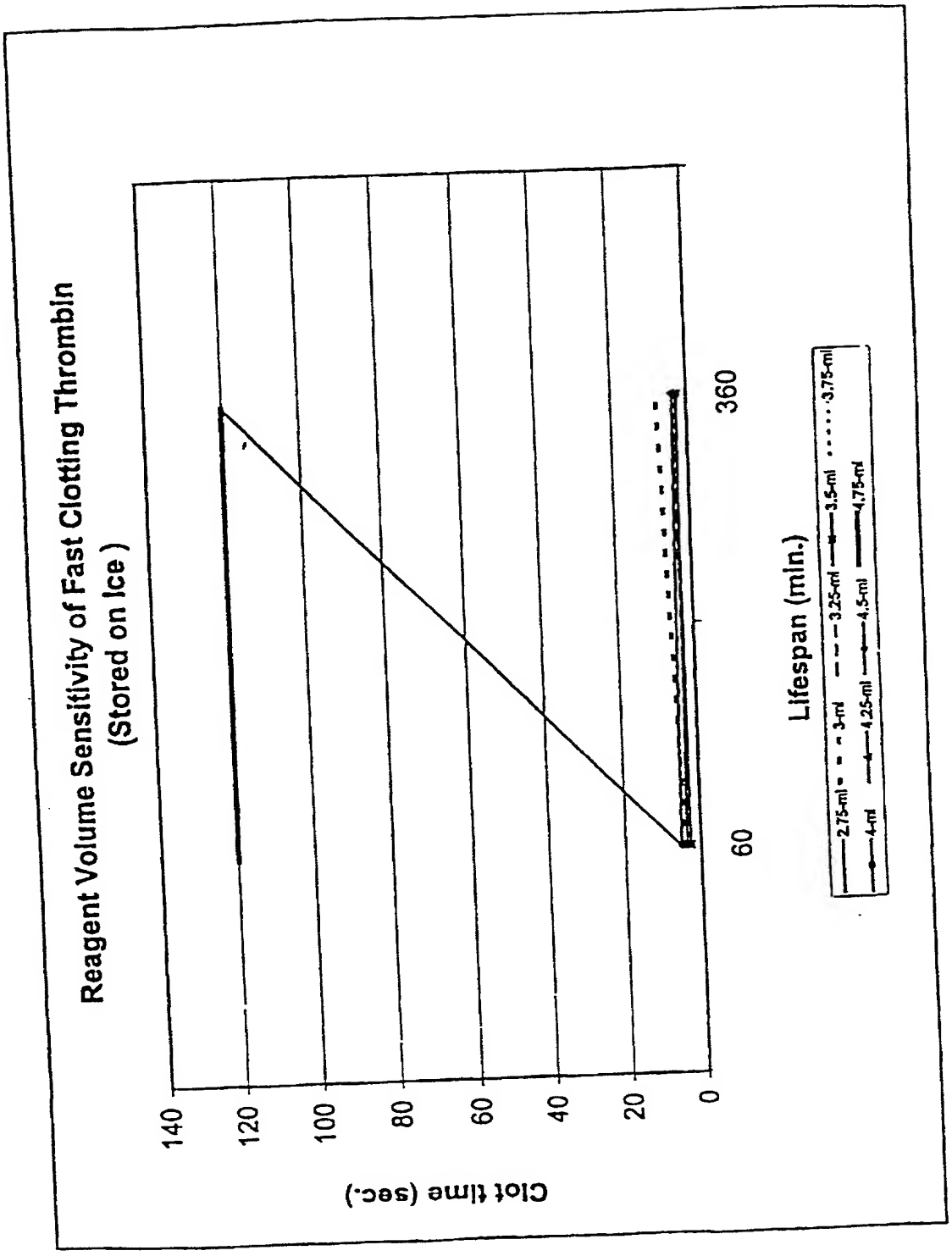

Reagent Volume Sensitivity of Fast Clotting Thrombin
(Stored on Ice )
Clot time (sec.)
140
120
100
80
60
40
20
0
60
360
Lifespan (min.)
2.75-ml
3-ml
3.25-ml
3.5-ml
3.75-ml
4-ml
4.25-ml
4.5-ml
4.75-ml

Figure 13

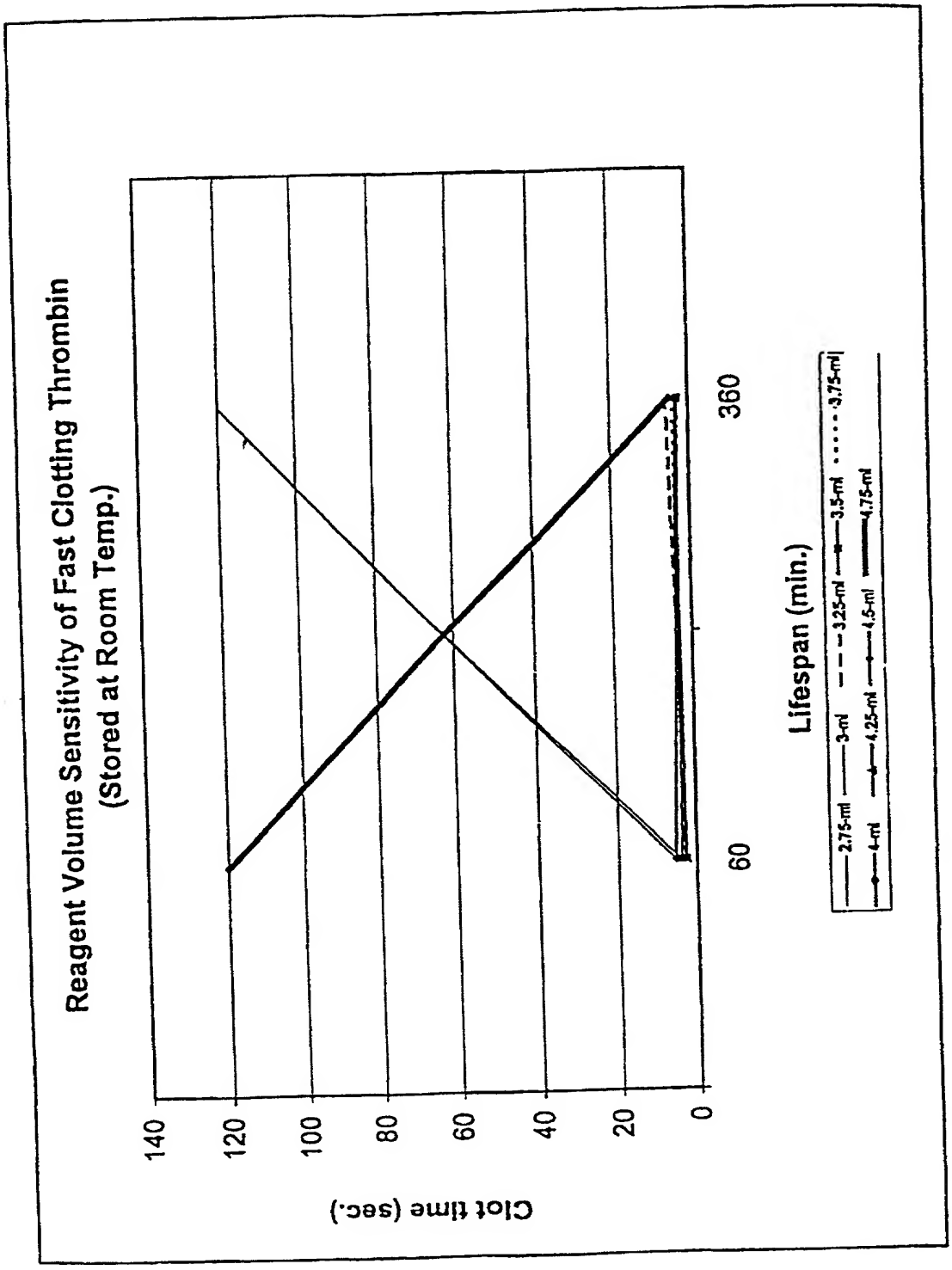

Reagent Volume Sensitivity of Fast Clotting Thrombin
(Stored at Room Temp.)
Clot time (sec.)
140
120
100
80
60
40
20
0
60
360
Lifespan (min.)
2.75-ml
3-ml
3.25-ml
3.5-ml
3.75-ml
4-ml
4.25-ml
4.5-ml
4.75-ml

Figure 14

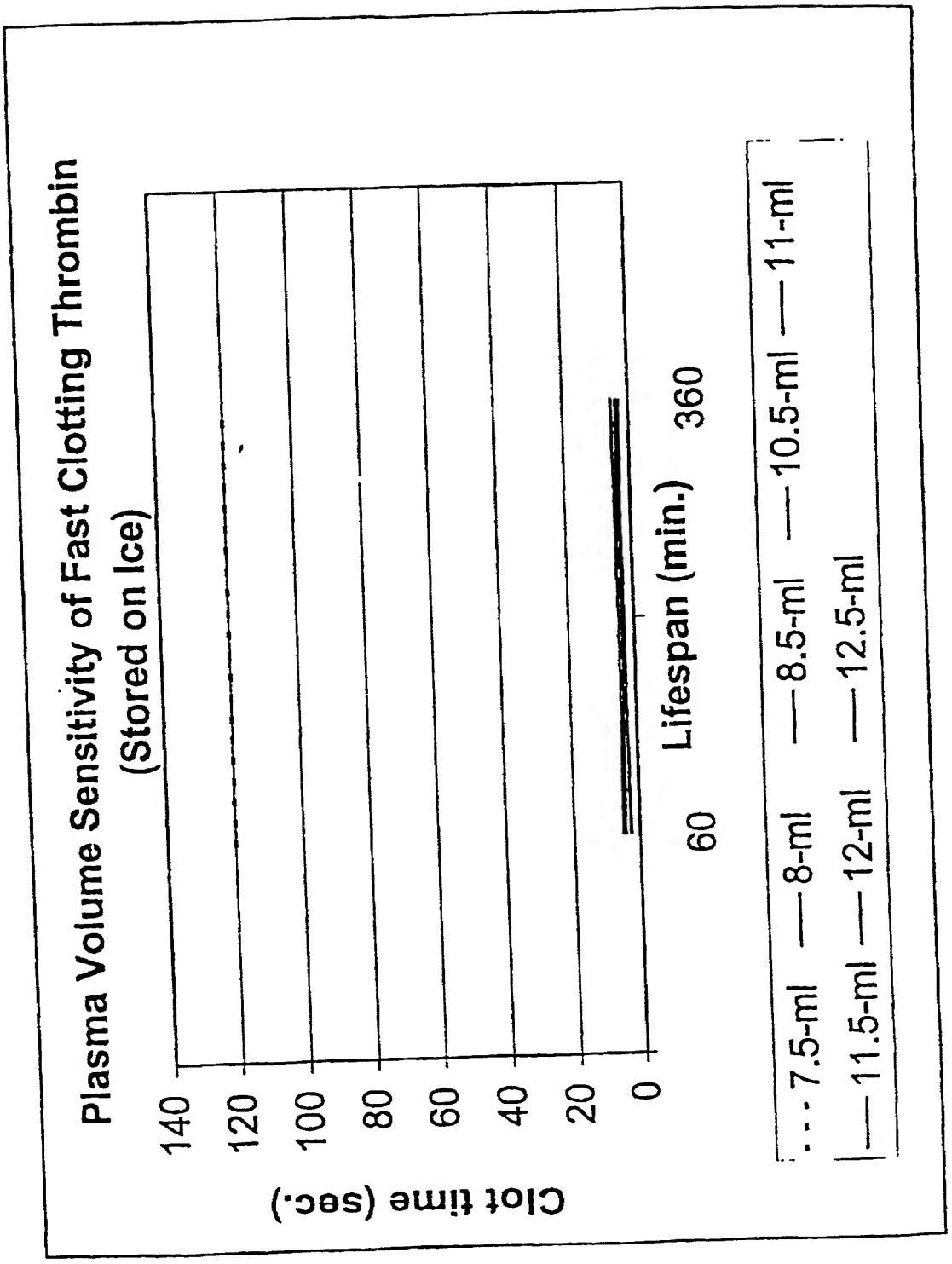

Plasma Volume Sensitivity of Fast Clotting Thrombin
(Stored on Ice)
Clot time (sec.)
140
120
100
80
60
40
20
0
60
Lifespan (min.)
360
7.5-ml
8-ml
8.5-ml
10.5-ml
11-ml
11.5-ml
12-ml
12.5-ml

Figure 15

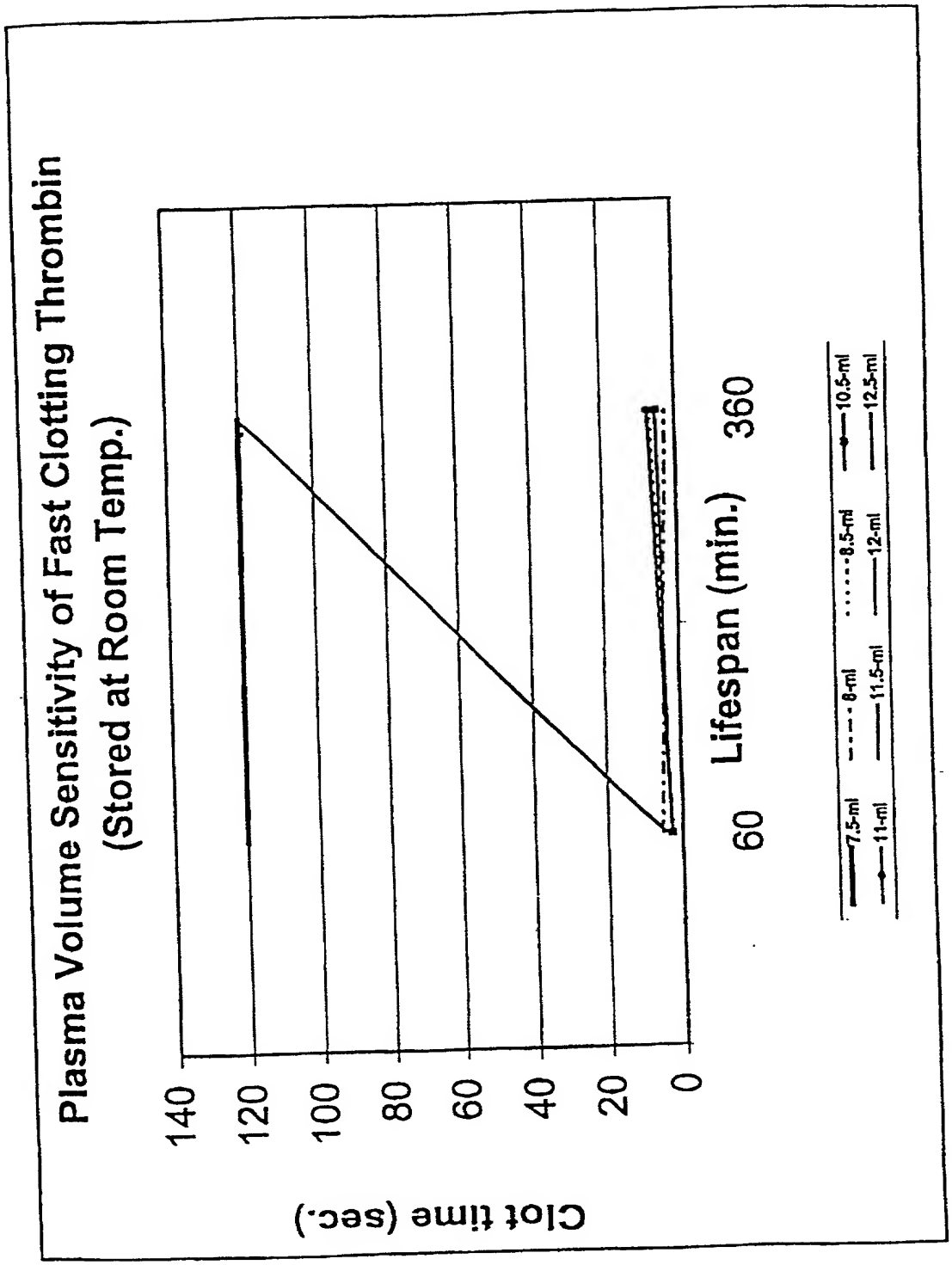

Plasma Volume Sensitivity of Fast Clotting Thrombin
(Stored at Room Temp.)
Clot time (sec.)
140
120
100
80
60
40
20
0
60
Lifespan (min.)
360
7.5-ml
8-ml
8.5-ml
10.5-ml
11-ml
11.5-ml
12-ml
12.5-ml

Figure 16

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# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

[XXX] Declaration Submitted with Initial Filing OR [ ] Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

| | |
|---|---|
| **Attorney Docket Number** | 31120-pa |
| **First Named Inventor** | Coelho, P., et al. |
| ***COMPLETE IF KNOWN*** | |
| Application Number | / |
| Filing Date | |
| Group Art Unit | |
| Examiner Name | |

**As a below named inventor, I hereby declare that:**

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Autologous Thrombin

*(Title of the Invention)*

the specification of which

[ ] is attached hereto

OR

[XX] was filed on (MM/DD/YYYY) June 2, 2000 as United States Application Number or PCT International Application Number PCT/US00/11865 and was amended on (MM/DD/YYYY) October 25, 2000 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application Number(s) | Country | Foreign Filing Date (MM/DD/YYYY) | Priority Not Claimed | Certified Copy Attached? YES | NO |
|---|---|---|---|---|---|
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

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# DECLARATION — Utility or Design Patent Application

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] OR ☑ Correspondence address below

Bernhard Kreten
**Name**

**Address** 77 Cadillac Drive, Suite 245

| Sacramento | California | 95825 |
|---|---|---|
| **City** | **State** | **ZIP** |

| United States | (916) 921-6181 | (916) 921-9213 |
|---|---|---|
| **Country** | **Telephone** | **Fax** |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**NAME OF SOLE OR FIRST INVENTOR :** ☐ A petition has been filed for this unsigned inventor

1-00

| **Given Name** (first and middle [if any]) Philip H. | **Family Name or Surname** Coelho |
|---|---|

**Inventor's Signature** Philip H. Coelho **Date** Nov 13, 2001

| El Dorado Hills | California | United States | United States CA |
|---|---|---|---|
| **Residence: City** | **State** | **Country** | **Citizenship** |

**Mailing Address** 121 Giotto Way

| El Dorado Hills | California | 95762 | United States |
|---|---|---|---|
| **City** | **State** | **ZIP** | **Country** |

**NAME OF SECOND INVENTOR:** ☐ A petition has been filed for this unsigned inventor

2-01

| **Given Name** (first and middle [if any]) Phil | **Family Name or Surname** Kingsley |
|---|---|

**Inventor's Signature** Phil Kingsley **Date** 11/14/01

| Mather | California | United States | United States CA |
|---|---|---|---|
| **Residence: City** | **State** | **Country** | **Citizenship** |

**Mailing Address** 4345 Gorham Way

| Mather | California | 95655 | United States |
|---|---|---|---|
| **City** | **State** | **ZIP** | **Country** |

☑ Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

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# DECLARATION

**ADDITIONAL INVENTOR(S)**
**Supplemental Sheet**
Page 1 of 2

| | | | |
|---|---|---|---|
| **Name of Additional Joint Inventor, if any:** | ☐ A petition has been filed for this unsigned inventor | | |
| Given Name (first and middle [if any]) | Family Name or Surname | | |
| Jim | Brausch | | |
| **Inventor's Signature** | | | **Date** 10/31/01 |
| 6875 Villa Juarez Circle **Residence: City** Sacramento | **State** CA | **Country** USA | **Citizenship** US CA |
| **Mailing Address** 6875 Villa Juarez Circle | | | |
| **Mailing Address** | | | |
| **City** Sacramento | **State** CA | **ZIP** 95828 | **Country** US |

| | | | |
|---|---|---|---|
| **Name of Additional Joint Inventor, if any:** | ☐ A petition has been filed for this unsigned inventor | | |
| Given Name (first and middle [if any]) | Family Name or Surname | | |
| James H. | Godsey | | |
| **Inventor's Signature** James H Godsey | | | **Date** 11-14-01 |
| 101 Summer Shade Court **Residence: City** Folsom | **State** CA | **Country** USA | **Citizenship** US CA |
| **Mailing Address** 101 Sumer Shade Court | | | |
| **Mailing Address** | | | |
| **City** Folsom | **State** CA | **ZIP** 95630 | **Country** USA |

| | | | |
|---|---|---|---|
| **Name of Additional Joint Inventor, if any:** | ☐ A petition has been filed for this unsigned inventor | | |
| Given Name (first and middle [if any]) | Family Name or Surname | | |
| Gail | Rock | | |
| **Inventor's Signature** Gail Rock | | | **Date** 7 OCT 2001 |
| 270 Sandridge Road **Residence: City** Ottawa | **State** Ontario | **Country** Canada | **Citizenship** Canada CAX |
| **Mailing Address** 270 Sandridge Road | | | |
| **Mailing Address** | | | |
| **City** Ottawa | **State** Ontario | **ZIP** K1L 5A2 | **Country** Canada |

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# DECLARATION

**ADDITIONAL INVENTOR(S)**
**Supplemental Sheet**
Page 2 of 2

| **Name of Additional Joint Inventor, if any:** | ☐ A petition has been filed for this unsigned inventor | | |
|---|---|---|---|
| Given Name (first and middle [if any]) | Family Name or Surname | | |
| Trista K. | Madsen | | |
| **Inventor's Signature** Trista K. Madsen | | | **Date** 11-1-01 |
| **Residence: City** 8782 Los Encantos Circle | **State** CA | **Country** USA | **Citizenship** US |
| **Mailing Address** 8782 Los Encantos Circle | | | |
| **Mailing Address** | | | |
| **City** Elk Grove | **State** CA | **ZIP** 95624 | **Country** USA |

| **Name of Additional Joint Inventor, if any:** | ☐ A petition has been filed for this unsigned inventor | | |
|---|---|---|---|
| Given Name (first and middle [if any]) | Family Name or Surname | | |
| Sona B. | Frausto | | |
| **Inventor's Signature** Sona B. Frausto Sona B. Frausto 10/16/01 | | | **Date** 10/12/01 |
| **Residence: City** 7954 Graylodge Court | **State** CA | **Country** USA | **Citizenship** US |
| **Mailing Address** 7954 Graylodge Court | | | |
| **Mailing Address** | | | |
| **City** Sacramento | **State** CA | **ZIP** 95828 | **Country** USA |

| **Name of Additional Joint Inventor, if any:** | ☐ A petition has been filed for this unsigned inventor | | |
|---|---|---|---|
| Given Name (first and middle [if any]) | Family Name or Surname | | |
| | | | |
| **Inventor's Signature** | | | **Date** |
| **Residence: City** | **State** | **Country** | **Citizenship** |
| **Mailing Address** | | | |
| **Mailing Address** | | | |
| **City** | **State** | **ZIP** | **Country** |

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# POWER OF ATTORNEY OR AUTHORIZATION OF AGENT

| | |
|---|---|
| **Application Number** | |
| **Filing Date** | |
| **First Named Inventor** | Coelho, Philip H., et al. |
| **Title** | Autologous Thrombin |
| **Group Art Unit** | |
| **Examiner Name** | |
| **Attorney Docket Number** | 31120-pa |

I hereby appoint:

☐ Practitioners at Customer Number [ ] → *Place Customer Number Bar Code Label here*

***OR***

☒ Practitioner(s) named below:

| Name | Registration Number |
|---|---|
| Bernhard Kreten | 27,037 |
| | |
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as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

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| ☐ Firm *or* Individual Name | | | | | |
| Address | | | | | |
| Address | | | | | |
| City | | State | | Zip | |
| Country | | | | | |
| Telephone | | Fax | | | |

I am the:

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☐ Assignee of record of the entire interest. See 37 CFR 3.71.
*Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).*

**SIGNATURE of Applicant or Assignee of Record**

| | |
|---|---|
| Name | Phil Kingsley |
| Signature | |
| Date | 11/14/01 |

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 8 forms are submitted

Please type a plus sign (+) inside this box → [X]

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# POWER OF ATTORNEY OR AUTHORIZATION OF AGENT

| | |
|---|---|
| **Application Number** | |
| **Filing Date** | |
| **First Named Inventor** | Coelho, Philip H., et al. |
| **Title** | Autologous Thrombin |
| **Group Art Unit** | |
| **Examiner Name** | |
| **Attorney Docket Number** | 31120-pa |

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**SIGNATURE of Applicant or Assignee of Record**

| | |
|---|---|
| Name | James H. Godsey |
| Signature | James H Godsey |
| Date | 11-14-01 |

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Please type a plus sign (+) inside this box → [X]

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| | |
|---|---|
| Name | Philip H. Coelho |
| Signature | Philip H. Coelho |
| Date | Nov 13, 2001 |

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| **Attorney Docket Number** | 31120-pa |

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*Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).*

**SIGNATURE of Applicant or Assignee of Record**

| | |
|---|---|
| Name | ThermoGenesis Corp., By: Philip H. Coelho, Its: Chief Executive Officer |
| Signature | Philip H. Coelho |
| Date | Nov. 13, 2001 |

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| | |
|---|---|
| Name | Trista K. Madsen |
| Signature | |
| Date | 11-1-01 |

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**SIGNATURE of Applicant or Assignee of Record**

| | |
|---|---|
| Name | Sona B. Frausto |
| Signature | Sona B. Frausto Sona B Frausto |
| Date | 10/12/07 10/16/07 |

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**SIGNATURE of Applicant or Assignee of Record**

| | |
|---|---|
| Name | Gail Rock |
| Signature | Gail Rock |
| Date | 2 Oct 2001 |

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|---|---|
| Name | Jim Brausch |
| Signature | |
| Date | 10-31-2001 |

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